

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



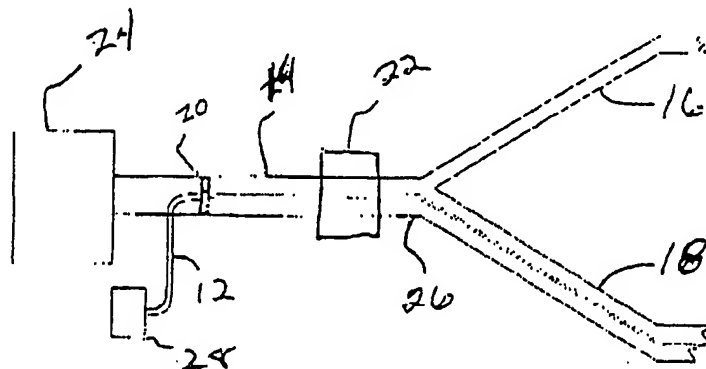
(43) International Publication Date
25 September 2003 (25.09.2003)

PCT

(10) International Publication Number
WO 03/078972 A1

- (51) International Patent Classification⁷: **G01N 15/14**, B07C 5/00
- (74) Agent: **LITZINGER, Jerrold, J.**; Sentron Medical, Inc., 4445 Lake Forest Drive, Suite 600, Cincinnati, OH 45242 (US).
- (21) International Application Number: PCT/US03/07173
- (22) International Filing Date: 10 March 2003 (10.03.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/264,343 14 March 2002 (14.03.2002) US
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicant (*for all designated States except US*): **MI-CRONICS, INC.** [US/US]; 8463 154th Avenue NE, Building F, Redmond, WA 98053 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **WEIGL, Bernhard, H.** [AT/US]; 5530 Canfield Place N., Seattle, WA 98103 (US). **BARDELL, Ronald, L.** [US/US]; 8500 148th Avenue NE, Redmond, WA 98052 (US). **HAYENGA, Jon, W.** [US/US]; 5310 240th Ave. NE, Redmond, WA 98052 (US).
- Published:
— with international search report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: RIBBON FLOW CYTOMETRY AND CELL SORTING



(57) Abstract: A microfluidic device for sorting cells is described. The device includes an input channel (12), a primary channel (14), at least two branch channels (16, 18) which connect with the primary channel (14) at a junction, and a sheath injector (20) positioned upstream from the junction. Sample solution, which may contain a population of cells, can be entered into the input channel (12) and hydrodynamically focused into a sample ribbon. The device employs a system for directing fluid flow, and particularly the flow of the sample ribbon, into a branch channel (16, 18) based on a detected cell feature. The system can employ a variety of sorting techniques to change or direct the flow of cells into a particular branch channel.

WO 03/078972 A1

RIBBON FLOW CYTOMETRY AND CELL SORTING

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application claims priority from U.S. Provisional Patent Application No. 60/364,343, filed March 14, 2002, entitled "Microfluidic Systems and Methods".

FIELD OF THE INVENTION

10

 This invention relates generally to microfluidic devices for performing analytic testing, and, in particular, to a microfluidic device and method for hydrodynamically focusing and sorting cells.

15

BACKGROUND OF THE INVENTION

 Microfluidic devices are becoming increasingly popular for performing analytical testing. Tools developed by the semiconductor industry for miniaturizing electronics enable fabrication and inexpensive mass production of intricate fluid systems. Microfluidic systems are increasingly utilized in performing a variety of analytical techniques for the acquisition of information in multiple disciplines, including the medical field, life sciences, and drug discovery and development.

25 There are many different ways to manufacture microfluidic devices, including traditional lithographic techniques, soft lithography, and laminate technologies. In laminate fabrication the device consists of layers of material or lamina that have been cut, such as by a laser or stamped, into the desired shape and then held together with some form of adhesive, most commonly pressure-sensitive or thermally-activated adhesive. Mylar plastic is commonly used, although other materials such as glass and polydimethylsiloxane (PDMS) have also been successfully incorporated into laminate devices. Microfluidic device construction may include a multi-layer laminated structure where each layer has

30

channels and structures fabricated from a laminate material, forming microscale voids or channels where fluids flow. A microscale channel is generally defined as a fluid passage with at least one internal cross-sectional dimension that is less than 500 micrometers and typically between about 0.1 micrometers and about 500 micrometers. Either external pressurized fluid forced into the laminate or structures located within the laminate affect the control and pumping of fluids through these channels.

Under microfluidic conditions, fluids usually flow in a very predictable, laminar fashion, thereby allowing multiple fluids to flow next to each other in the same channel without turbulent mixing or the need for physical separation by a membrane. Smaller particles typically diffuse quickly across the boundary layer, whereas large molecules and particles, such as cells, typically diffuse only minimally.

U.S. Patent No. 5,716,852 teaches a method for analyzing the presence and concentration of small particles in a flow cell using laminar flow and diffusion principles, and is incorporated by reference herein in its entirety for all purposes. Described is a channel cell system for detecting the presence of analyte particles in a sample stream using a laminar flow channel having at least two inlet means which provide an indicator stream and a sample stream, where the laminar flow channel has a depth sufficiently small to force laminar flow of the streams and length sufficient for diffusion of particles of the analyte into the indicator stream to form a detection area, and having an outlet out of the channel to form a single mixed stream. This device, which is known as a T-Sensor, may contain an external detecting means for detecting diffusion boundaries in the indicator stream. This detecting means may be provided by any means known in the art, including optical means such as optical spectroscopy, or absorption spectroscopy of fluorescence.

Flow cytometry is a method for simultaneously measuring light scatter, fluorescence, and absorption properties of single cells or particles as they flow in fluid suspension. Developed flow cytometric methods allow determination of

cellular features such as size, cell membrane permeability, intracellular pH, and the levels of cellular components such as nucleic acids, protein, surface receptor expression, and intracellular calcium levels. Some systems allow sorting or separation of particles or cells according to the properties exhibited. Flow
5 cytometry is increasingly used in basic research as well as in clinical, biological and environmental applications.

Presently, the state of the art in flow cytometry and cell sorting technology uses a hydrodynamically focused core stream, which is focused in two
10 dimensions to roughly the size of a cell in the dimensions orthogonal to flow. This produces a single-file cell stream, which can be presented to a light scatter, fluorescent detector, or image based cell detector system. Cell sorting technology generally combines piezoelectric drop generation and electrostatic deflection. In this manner, cells are partitioned into micro droplets and each
15 micro droplet is charged so that it may be electrostatically deflected into separate bins for sorting. This scheme suffers from the significant limitation that the detectors may only detect and direct one cell at a time. In order to process large quantities of cells, the fluidic systems must be run in a manner such that fluid passes the detectors at extremely high speeds. Fluid must pass the detector at
20 speeds from one to tens of meters per second in order to achieve necessary throughput for some applications. Because of these limitations, searching for rare cells or particles, such as cancer cells within a liquid preparation, for stem cells, fetal cells, or other can be inefficient, time consuming and costly.

25 SUMMARY OF THE INVENTION

The invention provides a microfluidic device and method for hydrodynamically focusing a sample solution into a ribbon and sorting cells based on a desired feature.

30 A microfluidic device for sorting cells is provided herein. The device includes an input channel, a primary channel, at least two branch channels which meet with the primary channel at a junction, and a sheath injector positioned

upstream from the junction. Sample solution, which may contain a population of cells, can be entered into the input channel and hydrodynamically focused into a sample ribbon. The device also consists of a particle diversion system for directing fluid flow, and particularly the flow of the sample ribbon, into a branch channel based on a detected cell feature.

Furthermore, a method of sorting cells in a microfabricated structure is described. The method consists of hydrodynamically focusing a population of cells into a sample ribbon, flowing the ribbon through a channel, determining the presence or amount of label on each cell, and diverting cells into a particular branch channel based on the presence or amount of label on each cell.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates a side view of a microfluidic device for sorting cells according to the invention;

FIG. 2 shows a three dimensional view of a microfluidic device for sorting cells according to the invention;

FIG. 3 is a side view of the sheath injector of the present invention;

FIG. 4 is a top view of the sheath injector of FIG. 3;

FIGS. 5A and 5B are schematic view of a portion of a sorting unit according to an embodiment of the invention engaged in directing flow of a sample ribbon using valves,

FIGS. 6A and 6B are schematic view of a portion of a sorting unit according to an embodiment of the invention engaged in directing flow of a sample ribbon using fluid displacement;

FIG. 7 is a schematic view of an alternative embodiment of a sorting unit according to the present invention; and

FIG. 8 is a schematic view of another alternative embodiment of a sorting unit according to the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The term "microfluidic" is generally defined as a substrate having a fluid passage with at least one internal cross-sectional dimension that is less than 500 micrometers and typically between about 0.1 micrometers and about 500 micrometers. The term "channel" as used herein, refers to a microfluidic channel and describes fluid elements dimensioned so that flow therein is substantially laminar. The terms "top", "bottom" and "side" refer to the orientation in the drawings, which is not necessarily the orientation of the members in operation.

As used herein, term "cartridge" refers to a microfluidic device, which is typically, but not necessarily, disposable and which can be coupled with measurement, pumping, electronic, fluidic or other apparatus. The cartridge can be microfabricated using a variety of methods, including without limitation, traditional lithographic techniques, soft lithography, laminate technologies, etc. For example, the cartridge can be microfabricated from any moldable, machinable or etchable substrate. The term machining as used herein includes, without limitation printing, stamping cutting and laser ablating. The cartridge can be formed in a single sheet, in a pair of sheets sandwiched together, or in a plurality of sheets laminated together. The term "sheet" refers to any solid substrate, flexible or otherwise. The channels can be etched in a silicon substrate and covered with a cover sheet, which can be a transparent cover sheet. In a laminated embodiment, the channel walls are defined by removing material from at least one sheet, thus creating channels and voids, and positioning additional sheets on either side of the altered sheets. Any of the layers can contain fluid channels. In some cases the channel is simply a hole (or

fluid via) to route the fluid to the next fluid laminate layer. Any two adjacent laminate layers may be bonded together to form a more complex single part.

As used herein, the term "flow" refers to any type of movement of a liquid or solid through a microfluidic device or in a method according to the invention.

5 Under microfluidic conditions fluid flow is characterized by a low Reynolds number and is laminar, with any mixing of adjacent fluids being mainly by diffusion. Flow also includes, without limitation, any fluid stream as well as any material, cells or particles moving with, within, or against the fluid stream. Any type of force may be applied in order to provide a flow, including without
10 limitation, pressure, capillary action, magnetic and electromagnetic force, electrophoresis, dielectrophoresis, electroosmosis, optical tweezers, and any combinations thereof.

The members of the flow system of this invention that are "connected" are
15 fluidically connected. The term "between" refers to the fluidic positioning, which does not necessarily correspond to the geometric positioning.

As used herein, the term "cell" means any cell, virus, material or particle having a microscopic size similar to a biological cell. A biological cell includes
20 without limitation, any prokaryotic, eukaryotic, bacterial, fungal, animal, plant, algae cell or otherwise. The size of a cell typically ranges from about 0.1 to 120 microns in diameter, typically being about 1 to 50 microns in diameter. Cells can be living or dead, charged or uncharged. Cells may be spherical or non-spherical, including without limitation, elongated, flattened, deformed and any
25 other asymmetrical cells. The term "cell" also includes microscopic beads, liposomes, emulsions, cellular components and complexes organelles or any other cell-sized particles.

The term "label" refers to a molecule or composition of molecules that is
30 detectable by optical, spectroscopic, photochemical, biochemical, immunological, chemical or magnetic means. Labels can be specifically targeted to selected cells, but need not be. Such markers or labels include, but are not limited to, colored, radioactive, fluorescent, ultraviolet, or magnetic molecules or particles

conjugated to antibodies or other molecules or particles known to bind to cells or cellular components. Antibodies are often used as label components because of their ability to target specific cell types. Other reactive label components that can serve as alternatives to antibodies include, but are not limited to, genetic probes, dyes, fluorochromes, proteins, peptides, amino acids, sugars, polynucleotides, enzymes, coenzymes, cofactors, antibiotics, steroids, hormones or vitamins. The label often generates a measurable signal, which can be detected with or without some kind of stimulatory event and can be used to detect the presence of bound label and possibly quantitate the amount of bound label in a sample.

Furthermore, the label may be a detectable intrinsic property of the cell, such as cell size or morphology, which is detectable, for example, by measuring light scattering characteristics. The label may be directly detectable or indirectly detectable or operate in conjunction with another label. For further examples of labels see those listed in Handbook of Fluorescent Probes and Research Chemicals, 9th Ed., Molecular Probes, Inc., Eugene, Oregon.

As used herein, the term "labeling" refers to the process of affixing or conjugating a label to cells, allowing, sometimes after further processing, those cells to be separated from a heterogeneous suspension and/or detected, analyzed or counted in accordance with the present invention.

The terms cell "feature" and cell "characteristic" may be used interchangeably and refer to any property of a cell that is detectable by labeling. Non-limiting examples include size, shape, morphology, presence or amount of a particular cellular molecule (e.g., protein, nucleotide, lipid, carbohydrate, hormone, etc) located internally, on the exterior of the cell, or any combination thereof, expression of a gene or protein, cell permeability, affinity for a dye, cell cycle stage, number of mitotic divisions, or any combination thereof.

A sorting unit permits sorting of cells according to the invention and consists of an input channel, a primary channel, at least two branch channels, a sheath injector, and an interrogation region. A sorting unit further consists of a cell diversion system, which allows the flow of cells to change direction and enter

one or more of the branch channels, depending on a signal received in connection with an examination in the interrogation region. An input channel is a channel for receiving sample solution and is connected with the sheath injector. Sample solution is fluid that flows through the input channel and sheath injector, and is hydrodynamically focused in the primary channel. Sample solution typically, but not necessarily, contains a population of cells.

The primary channel permits the flow of cells past the interrogation region and allows for sorting according to the invention. The primary channel consists of a first end connected with a sheath solution reservoir and a second end connected with at least two branch channels that meet at a junction.

A branch channel is a channel that is connected with a primary channel. Typically, a branch channel receives cells depending on the cell feature of interest as detected in the interrogation region and sorted by the cell diversion system. A branch channel may be connected with other channels to permit additional sorting. Alternatively, a branch channel may terminate with a well or reservoir to allow collection or disposal of the cells.

A junction is an area where the primary channel and at least two branch channels join together. It is at the junction where the flow of cells can change direction to enter one or more other channels, e.g., a branch channel, depending on the signal received in connection with an examination in the interrogation region. The interrogation region may be positioned along the primary channel either at, proximate to, or upstream of the junction.

The sheath injector is typically positioned along the primary channel and is connected with an input channel. The sheath injector permits introduction and hydrodynamic focusing of sample solution in the primary channel. The sheath injector according to the invention permits hydrodynamic-focusing, for example, in one dimension permitting the production of a sample ribbon.

A sample ribbon is produced, for example, by hydrodynamically focusing sample solution in one dimension. The width of a sample ribbon is typically greater than the height. In one embodiment of the present invention, dimensions of a sample ribbon consists of a formation of cells with a height at least that of a single cell and a width of at least two cells. The ribbon may be at least one cell in length and may extend further depending the amount of cells contained within the sample solution.

The interrogation region is typically located along a portion of the primary channel where cells to be sorted are examined for sorting on the basis of a feature that is predetermined. In an embodiment according to the present invention, a portion of the sample ribbon is examined as it flows through the interrogation region, allowing examination of a plurality of cells at one time. The term plurality refers to a number equal to or greater than two. The predetermined feature is detected or measured, for example, by testing for the presence or amount of a label. For example, the interrogation region can be connected with a recognition apparatus.

The recognition apparatus allows identification, detection, and/or sometimes the quantification of at least one cell feature as determined by a labels or plurality of labels. For example, the recognition apparatus may consist of one or more microscopes, cameras, imagers, diodes, light stimulating devices (e.g. lasers, lamps, etc.), photomultiplier tubes, and processors (e.g. computers and software), and any combinations thereof, which cooperate to detect a signal representative of a cell feature or label, and enable the determination and direction of the sorting of cells into a particular branch channel.

The cell diversion system is a means by which the flow of cells can change direction to enter one or more of the branch channels, depending on a signal received in connection with an examination in the interrogation region. A cell diversion system can employ a variety of sorting techniques to change or direct the flow of cells into a particular branch channel, including without limitation, electric, electroosmotic, valve, magnetic, fluid displacement, pressure, etc.

Figure 1 shows a side view of a sorting unit 10, according to an exemplary embodiment of the invention. A sorting unit 10 permits sorting of cells according to the invention and consists of an input channel 12, a primary channel 14, at least two branch channels 16, 18, a sheath injector 20, and an interrogation region 22. The primary channel 14 consists of a first end typically connected with a sheath fluid reservoir 24, which can be located, for example, either within or separate from the same microfabricated substrate containing the primary channel 14. The primary channel 14 further consists of a second end connected with at least two branch channels 16, 18, which connect at a junction 26. An interrogation region 22 is positioned along the primary channel 14 and located between the junction 26 and the sheath injector 20. An input channel 12 is in communication with the primary channel 14 by being connected with the sheath injector 20. The input channel 12 is also connected with a sample fluid reservoir 28. A sorting unit further consists of a cell diversion system (not shown in Figure 1), which is typically located at, near, or proximate the junction. A cell diversion system allows the flow of cells to change direction and enter one or more of the branch channels, depending on a signal received in connection with an examination in the interrogation region.

20

Figure 2 illustrates a three dimensional view of a sorting unit 30, according to another exemplary embodiment of the invention. Sorting unit 30 of Figure 2 consists of an input channel 32, a primary channel 34, at least two branch channels 36, 38, a sheath injector 40, and an interrogation region 42. In order to illustrate the operation of sorting unit 30, a ribbon 44 is shown with sorting unit 30 in Figure 2. Sheath solution can be entered into a sheath fluid reservoir 46 and flowed through the sorting unit 30, including through the primary channel 34. Sample solution can be entered into the sample reservoir 48 and can flow through the input channel 32 and into the primary channel 34. Sheath solution can enter the primary channel 34 through the sheath injector 40. At the sheath injector 40, the sample solution 54 is surrounded by sheath solution 52 and focused into a sample ribbon 44, as can be seen in FIGS. 3 and 4. The sheath solution 52 forms two high pressure streams above and below the sample

30

solution 54, which is flowing at a lower pressure, and a sample ribbon 44 is formed. The sample ribbon 44 can be further focused geometrically at an entrance 56 to a narrower portion of the primary channel 34. The sample ribbon 44 flows through the primary channel 34, past the interrogation region 42 and to the junction 58. Near to the junction 58, a cell diversion system allows the flow of cells to change direction and enter one or more of the branch channels 36, 38, depending on a signal received in connection with an examination in the interrogation region 42.

One particular exemplary embodiment of the invention including a cell diversion system is shown in FIGS. 5A and 5B, where at least a portion of a sorting unit 60 is illustrated, including a portion of a primary channel 62 and a plurality of branch channels 64, 66, which connect at a junction 68. In this embodiment, valves 70, 72 positioned along the branch channels 64, 66 control the direction of sample ribbon 74 flow. Valves 70, 72 are interconnected to the interrogation region 76 through the recognition apparatus, computer, or other automated system to allow opening and closing of appropriate valves depending on whether a desired material is detected or not. For example, initially, the top valve 70 is in a closed status and the bottom valve 72 is in an open status to allow fluid, including a sample ribbon 74, to flow from the primary channel 62 through the junction 68 and into the bottom branch channel 66. When a desired label is detected in the interrogation region 76, the bottom valve 72 is closed and the top valve 70 is opened to allow the ribbon 74 to be directed into the top branch channel 64.

In another example of an embodiment of the present invention, other means of differentially restricting flow between two or more branch channels can be employed. For example, U.S. patent No. 5, 726, 404, assigned to Micronics, Inc. and incorporated by reference herein in its entirety for all purposes, describes valveless liquid microswitches and teaches a valveless method and apparatus for high speed switching of liquid flow between intersecting microchannels. By manipulating the pressures, fluid flow can be differentially

restricted in the absence of valves to allow the direction of the sample ribbon into one or more branch channels 64, 66.

Another exemplary embodiment of the invention including a cell diversion
5 system is illustrated in FIGS. 6A and 6B. Shown is at least a portion of a sorting
unit 80, including a portion of a primary channel 82 and a plurality of branch
channels 84, 86, which connect at a junction 88. In this embodiment, a sample
ribbon 90 may be directed into one or more branch channels 84, 86 by means of
fluid displacement. Shown here, a channel 92 is connected with the primary
10 channel 82 between the junction 88 and the interrogation region 94, with the
channel 92 allowing fluid to enter the primary channel 82 upstream from the
junction 88. Fluid can flow from the primary channel 82 and through the junction
88. Initially, no fluid enters the primary channel 82 from the channel 92, and due
to the geometric positioning of the primary channel 82 and branch channels 84,
15 86, a portion of the fluid, including the sample ribbon 90 flows into the bottom
branch channel 86. When a desired label is detected in the interrogation region
94, fluid can be entered into the primary channel 82 from the channel 92. The
entering fluid partially displaces fluid flowing through the primary channel 82, and
causes the sample ribbon 90 to be directed into the top branch channel 84.

20

Alternatively, in another example, the sample ribbon 90 may be directed
into a branch channel by negative fluid displacement where fluid is removed from
the primary channel 82 by channel 92. In this example, removal of fluid from the
primary channel 82 by fluid flowing into channel 92 decreases the volume of fluid
25 in the primary channel 82 and directs the sample ribbon 90 into the bottom
branch channel 86. When the removal of fluid from the primary channel 82 by
channel 92 is interrupted, the volume of fluid in the primary channel 82 increases
and the sample ribbon 90 is directed into the top branch channel 84.

30 Yet another exemplary embodiment of the invention including a cell
diversion system is shown in FIG. 7, where at least a portion of a sorting unit 100
is illustrated, including a portion of a primary channel 102 and a plurality of
branch channels 104, 106 which connect at a junction 108. In this embodiment,

at least a portion of the sample ribbon 110 may be directed into one or more branch channels 104, 106 by the application of magnetic force. A magnetic source 112 is shown. Cells can be selectively labeled, for example, where an attached label includes a magnetically responsive portion, such as a magnetic bead conjugated with an antibody. For example, initially, due to the geometric positioning of the primary channel 102 and branch channels 104, 106, a portion of the fluid, including the sample ribbon 110 flows into one of the branch channels, such as the bottom branch channel 106. When a magnetic force is applied, cells containing a magnetic label are flow in response to the applied force. For example, a magnetic force may be applied to direct magnetically labeled cells into the top branch channel 104, whereas those cells would otherwise flow into the bottom branch channel 106 in the absence of an applied magnetic force.

FIG. 8 illustrates yet another exemplary embodiment of the invention, where is shown at least a portion of a sorting unit 120, including a portion of a primary channel 122 and a plurality of branch channels 124, 166 which connect at a junction 128. In this embodiment, at least a portion of the sample ribbon 130 may be directed into one or more branch channels 124, 126 by the application of electrical field. An electrical field source 132 is shown, with arrows representing an electrical field. Cells flowing through the electrical field are directed into one or more branch channels 124, 126 in response to the interaction between the charge of the cell and the applied electrical field. For example, initially, due to the geometric positioning of the primary channel 122 and branch channels 124, 126, a portion of the fluid, including the sample ribbon flows into one of the branch channels, such as the bottom branch channel 126. When an electrical field is applied, cells can flow in response to the applied force, depending on the charge of the cell. For example, an electrical field may be applied to direct cells carrying a particular charge into the top branch channel 124, whereas those cells would otherwise flow into the bottom branch channel 126 in the absence of an applied electrical field.

Other embodiments of the invention including a cell diversion system can employ a variety of sorting techniques to change or direct the flow of cells into a

particular branch channel, including without limitation, electric, electroosmotic, other valve arrangements, other means of fluid displacement, pressure, etc.

While the sorting device described above utilizes a Y-shaped junction, this
5 is not necessarily required. Other types of junctions, including without limitation, T-shaped junctions or junctions formed by the intersection of more than 4 channels could be utilized for sorting according to the invention.

Additionally, while only a single sorting unit is illustrated in the above
10 figures, the invention is not limited to this single configuration. A sorting unit according to the embodiments of the present invention is readily integratable with other structures on the same microfabricated substrate. For example, embodiments according to the present invention may include a series of consecutively arranged sorting units useful for segregating different cells in a
15 population through successive sorting operations. Another embodiment according to the present invention may include a cartridge microfabricated to include one or more sorting units, or a series of consecutively arranged sorting units.

Other arrangements, configurations and methods should be readily
20 apparent to a person of ordinary skill in the art. Other embodiments, combinations and modifications of this invention will occur readily to those of ordinary skill in the art in view of these teachings. Therefore, this invention is to be limited only by the following claims, which include all such embodiments and modifications when viewed in conjunction with the above specification and
25 accompanying drawings.

What is claimed is:

1. A microfluidic device for sorting cells, comprising:
 - a. an input channel for receiving sample solution;
 - 5 b. a primary channel, with a first end of the primary channel connected with a sheath solution reservoir and a second end connected with at least two branch channels which connect at a junction;
 - c. a sheath injector positioned upstream from the junction and connected with the input channel such that sample solution entering the primary channel flows through the sheath injector and is
10 hydrodynamically focused into a ribbon compressed between at least two layers of sheath solution;
 - d. an interrogation region positioned between the junction and the sheath injector, where the interrogation region is coupled with a recognition apparatus for evaluating the cells in the ribbon according to at least one cell feature as the ribbon passes through the interrogation region; and
15
 - e. a cell diversion system responsive to the recognition apparatus and capable of directing fluid flow into a branch channel based on a detected feature.
20
2. The microfluidic device of claim 1, wherein the cell diversion system consists of at least one valve positioned along each branch channel, with no more than the valves of one branch channel being open at any
25 one time, and the status of the valves being determined through communication with the recognition apparatus.
3. The microfluidic device of claim 1, wherein the cell diversion system consists of a means for manipulating pressure within the branch
30 channels, such that flow within the branch channels is differentially restricted and the sample ribbon can be directed into a branch channel.

4. The microfluidic device of claim 1, wherein the cell diversion system consists of a channel for introducing fluid, said channel being positioned upstream from the junction, with introduction of fluid from said channel being determined through communication with the recognition apparatus.
5. The microfluidic device of claim 1, wherein the cell diversion system consists of a channel for removing fluid from the primary channel, said channel being positioned upstream from the junction, with introduction of fluid from said channel being determined through communication with the recognition apparatus.
6. The microfluidic device of claim 1, wherein the cell diversion system consists of a magnetic source positioned proximate to the junction, such that cells conjugated with a label having a magnetically responsive portion and flowing through the junction are directed into one or more branch channels in response to the magnetic source.
7. The microfluidic device of claim 1, wherein the cell diversion system consists of an applied electrical field positioned proximate to the junction, such that cells flowing through the electrical field are directed into one or more branch channels in response to the interaction between the charge of the cell and the applied electrical field.
8. The microfluidic device of claim 1, further comprising a means for geometrically focusing the sample ribbon.
9. The microfluidic device of claim 1, wherein each evaluated cell feature is made detectable by use of a label.
10. The microfluidic device of claim 1, where labeled cells emit a fluorescent signal, which is detected by the recognition apparatus consisting of an optical detection means.

11. A sorting unit contained within a cartridge, comprising:
- a. an input channel for receiving sample solution;
 - b. a primary channel, with a first end of the primary channel connected with a sheath solution reservoir and a second end connected with at least two branch channels which connect at a junction;
 - c. a sheath injector positioned upstream from the junction and connected with the input channel such that sample solution entering the primary channel flows through the sheath injector and is hydrodynamically focused into a ribbon compressed between at least two layers of sheath solution;
 - d. an interrogation region positioned between the junction and the sheath injector, where the interrogation region is coupled with a recognition apparatus for detecting labeled cells as the ribbon passes through the interrogation region; and
 - e. a cell diversion system responsive to the recognition apparatus and capable of directing fluid flow into a branch channel based on a detected feature.
12. The sorting unit of claim 11, wherein the cell diversion system consists of at least one valve positioned along each branch channel, with no more than the valves of one branch channel being open at any one time, and the status of the valves being determined through communication with the recognition apparatus.
13. The sorting unit of claim 11, wherein the cell diversion system consists of a means for manipulating pressure within the branch channels, such that flow within the branch channels is differentially restricted and the sample ribbon can be directed into a branch channel.
14. The sorting unit of claim 11, wherein the cell diversion system consists of a channel for introducing fluid, said channel being positioned upstream from the junction, with introduction of fluid from said channel

being determined through communication with the recognition apparatus.

5 15. The sorting unit of claim 11, wherein the cell diversion system consists of a channel for removing fluid from the primary channel, said channel being positioned upstream from the junction, with introduction of fluid from said channel being determined through communication with the recognition apparatus.

10 16. The sorting unit of claim 11, wherein the cell diversion system consists of a magnetic source positioned proximate to the junction, such that cells conjugated with a label having a magnetically responsive portion and flowing through the junction are directed into one or more branch channels in response to the magnetic source.

15 17. The sorting unit of claim 11, wherein the cell diversion system consists of an applied electrical field positioned proximate to the junction, such that cells flowing through the electrical field are directed into one or more branch channels in response to the interaction between the charge of the cell and the applied electrical field.

20

18. The sorting unit of claim 11, further comprising a means for geometrically focusing the sample ribbon upstream from the interrogation region.

25 19. A method of sorting cells in a microfluidic device, comprising the steps of:

- 30 a. hydrodynamically focusing a population of cells into a ribbon compressed between at least two layers of sheath solution;
- b. flowing the ribbon through a channel comprising an interrogation region;
- c. determining the presence or amount of label on each cell as it passes through the interrogation region; and

d. diverting cells into a first branch channel or a second branch channel based on the presence or amount of label on each cell.

5 20. The method of claim 19, further comprising the step of geometrically focusing the ribbon upstream from the sample ribbon.

21. The method of claim 19, wherein the cell diverting step includes positioning at least one valve along each branch channel, such that no more than the valves of one branch channel are capable of being open at any one time, the status of the valves being determined through
10 communication with the recognition apparatus.

22. The method of claim 19, wherein the cell diverting step includes means for manipulating pressure within the branch channels, such that flow within the branch channels is differentially restricted and the sample ribbon can be directed into a branch channel.
15

23. The method of claim 19, wherein the cell diverting step includes positioning a channel for introducing fluid upstream from the junction, with introduction of fluid from said channel being determined through
20 communication with the recognition apparatus.

24. The method of claim 19, wherein the cell diverting step includes positioning a channel for removing fluid from the primary channel upstream from the junction, with introduction of fluid from said channel being determined through communication with the recognition apparatus.
25

25. The method of claim 19, wherein the cell diverting step includes positioning a magnetic source proximate the junction, such that cells conjugated with a label having a magnetically responsive portion and flowing through the junction are directed into one or more branch channels in response to the magnetic source.
30

26. The method of claim 19, wherein the cell diverting step includes positioning an applied electrical field proximate to the junction, such that cells flowing through the electrical field are directed into one or more branch channels in response to the interaction between the charge of the cell and the applied electrical field.

Figure 1

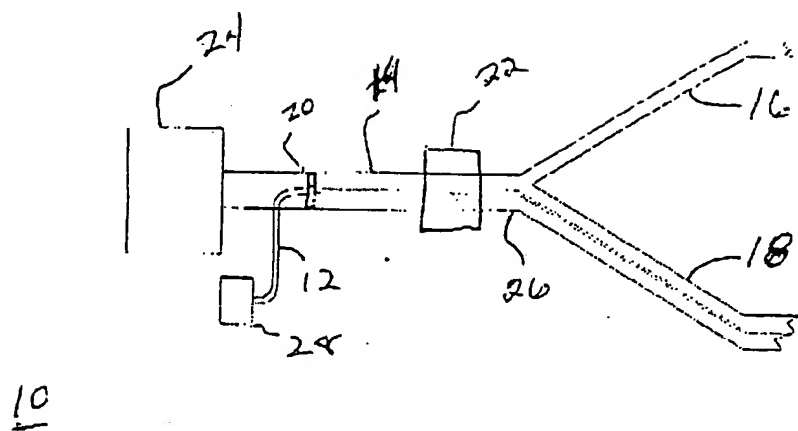


Figure 2

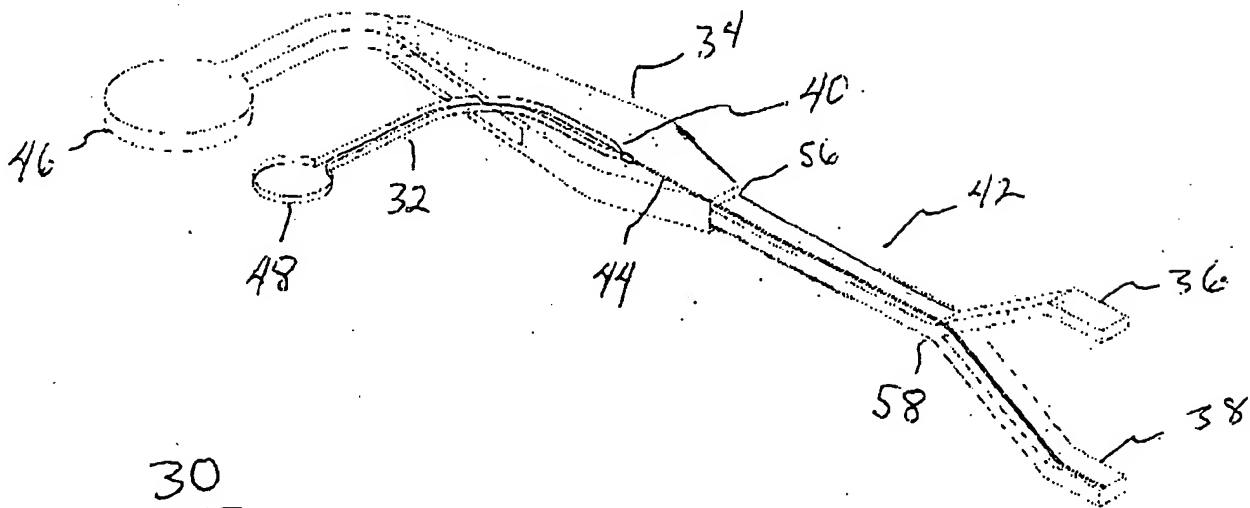


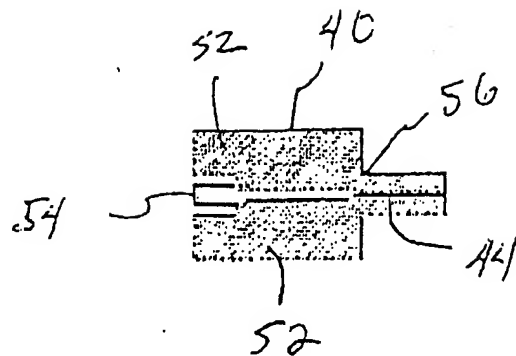
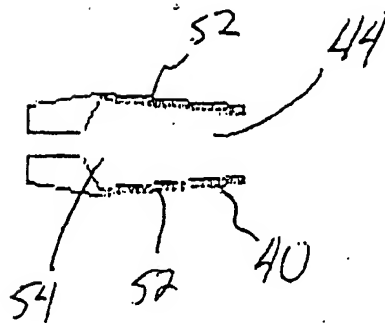
Figure 3Figure 4

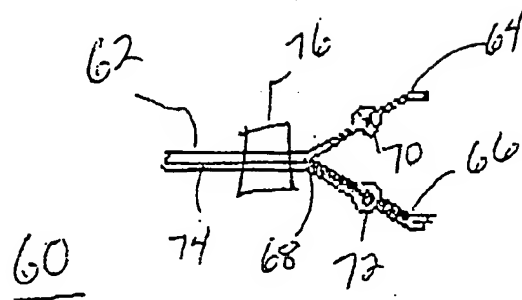
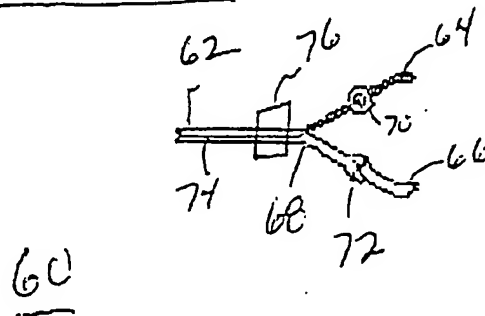
Figure 5aFigure 5b

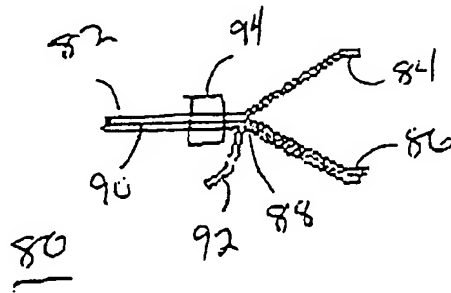
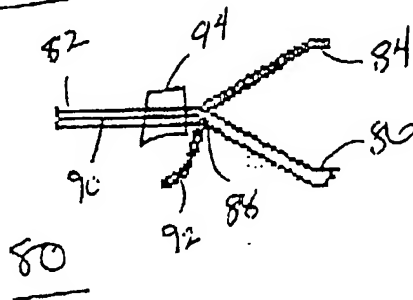
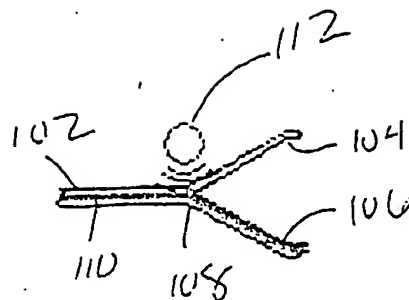
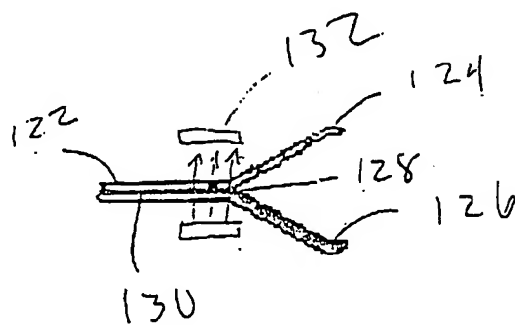
Figure 6aFigure 6b

Figure 7100Figure 8120

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N15/14 B07C5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 B01L G01N B07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 10267 A (BLANKENSTEIN GERT ; TECHNICAL UNIVERSITY OF DENMAR (DK)) 12 March 1998 (1998-03-12) abstract page 1, line 7-10 page 6, line 33 -page 16, line 20 page 22, line 34 -page 25, line 4 figures	1-26
X	DE 100 31 028 A (SMTECH BIOVISION HOLDING AG EC) 3 January 2002 (2002-01-03) the whole document	1-5, 8-15, 18-24

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * & * document member of the same patent family

Date of the actual completion of the international search

18 June 2003

Date of mailing of the international search report

30/06/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Smith-Hewitt, L

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 12903 A (SCHULZ TORSTEN ;INST PHYSIKALISCHE HOCHTECH EV (DE); KOEHLER JOHAN) 9 March 2000 (2000-03-09) the whole document ---	1-5, 8, 11, 13-15, 18-20, 22
X	WO 02 10713 A (HONEYWELL INT INC) 7 February 2002 (2002-02-07) page 11, line 32 -page 12, line 10; figure 7 ---	1-4, 8-15, 18-20, 23, 24
A	US 6 332 540 B1 (JAMES PHILIP JOHN ET AL) 25 December 2001 (2001-12-25) column 2, line 40 -column 9, line 44 ---	1-26
A	WO 99 61888 A (FU ANNE ;QUAKE STEPHEN (US); ARNOLD FRANCES (US); CALIFORNIA INST) 2 December 1999 (1999-12-02) page 4, line 10 -page 31, line 4 ---	1-26
A	US 5 837 200 A (DIESEL EDGAR ET AL) 17 November 1998 (1998-11-17) the whole document ---	1-26
A	US 4 756 427 A (SCHUMANN JOHANNES ET AL) 12 July 1988 (1988-07-12) the whole document ---	1-26
A	WO 01 49412 A (RIGLER RUDOLF ;ACREO AB (SE); HOLM JOHAN (SE)) 12 July 2001 (2001-07-12) the whole document ---	3, 13, 22
A	WO 91 15750 A (CARRI MED LTD) 17 October 1991 (1991-10-17) the whole document -----	1-26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 03/07173

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9810267	A	12-03-1998	AT 211258 T	15-01-2002
			AU 4113297 A	26-03-1998
			DE 69709377 D1	31-01-2002
			DE 69709377 T2	14-08-2002
			WO 9810267 A1	12-03-1998
			DK 925494 T3	01-07-2002
			EP 0925494 A1	30-06-1999
			JP 2002503334 T	29-01-2002
			NO 991051 A	27-04-1999
			US 2003044832 A1	06-03-2003
			US 6432630 B1	13-08-2002
DE 10031028	A	03-01-2002	DE 10031028 A1	03-01-2002
			AU 6908701 A	08-01-2002
			WO 0201189 A1	03-01-2002
			EP 1295105 A1	26-03-2003
WO 0012903	A	09-03-2000	AU 2250700 A	21-03-2000
			DE 19847952 A1	23-03-2000
			WO 0012903 A1	09-03-2000
			EP 1108149 A1	20-06-2001
			JP 2002523709 T	30-07-2002
			US 6497252 B1	24-12-2002
WO 0210713	A	07-02-2002	AU 8307401 A	13-02-2002
			WO 0210713 A2	07-02-2002
US 6332540	B1	25-12-2001	EP 0974046 A1	26-01-2000
			WO 9845683 A1	15-10-1998
			JP 2001521439 T	06-11-2001
WO 9961888	A	02-12-1999	AU 4955799 A	13-12-1999
			CA 2333201 A1	02-12-1999
			EP 1190229 A2	27-03-2002
			JP 2002528699 T	03-09-2002
			WO 9961888 A2	02-12-1999
			US 6540895 B1	01-04-2003
			US 2002005354 A1	17-01-2002
US 5837200	A	17-11-1998	DE 19520298 A1	05-12-1996
			DE 59608103 D1	13-12-2001
			EP 0745682 A1	04-12-1996
			JP 8332074 A	17-12-1996
US 4756427	A	12-07-1988	AT 48477 T	15-12-1989
			CA 1256825 A1	04-07-1989
			DE 3574617 D1	11-01-1990
			EP 0177718 A2	16-04-1986
			JP 1833433 C	29-03-1994
			JP 61137062 A	24-06-1986
WO 0149412	A	12-07-2001	AU 2675601 A	16-07-2001
			WO 0149412 A1	12-07-2001
WO 9115750	A	17-10-1991	WO 9115750 A1	17-10-1991